

# AI-based Design Workflow of De-Novo Antigens by Epitope Scaffolding

De-novo protein design allows a wider exploration of protein folding space than directed evolution and has been profitably employed in several fields of biochemistry and medicine, as metalloproteins design and in-vitro diagnostics (IVD) [1]. Among other design tasks, de novo protein binders can now be obtained with high success-rate that are highly specific towards a series of biological targets, thanks to the recent advances in the AI field [3,4,5]. Despite that, IVD industry still heavily relies on antibodies, as they are cheaper, and their production is well standardized. Therefore, a de-novo antigen may virtually speed-up and support the production of better antibodies. In this work, we present a workflow to scaffold small proteins (~50AA) around a known antigen epitope to elicit higher immune response in host organisms. The workflow is divided in two cyclic phases: generation and validation, each one using task-specific AI-models. The generation phase starts by creating several backbones of small scaffolds via diffusion models around a chosen epitope, whose position and residues are held constant during the process. Subsequently, protein language models are used on such backbones to assign the sequences. The structure of the generated sequences is then predicted through in-silico folding models and the most promising sequences are screened in-silico for immunogenicity with MaSIF 1, a model evaluating protein-protein interaction sites based on geometric neural network. The methodology has been performed on one epitope of HPV-16, obtaining 6 different small proteins which are currently being screened in-vitro. The proposed workflow is fully generalizable to any epitope, and we envision that it can pose a novel methodology in the design of the de-novo antigens.

1 Chino, M., Di Costanzo, L.F., Leone, L. et al. "Designed Rubredoxin miniature in a fully artificial electron chain triggered by visible light". *Nat Commun* 14, 2368 (2023). DOI: 1.

2 Chu, A.E., Lu, T. & Huang, P.S. "Sparks of function by de novo protein design". *Nat Biotechnol* 42, 203–215 (2024). DOI: 2.

3 Watson, J.L., Juergens, D., Bennett, N.R. et al. "De novo design of protein structure and function with RFdiffusion". *Nature* 620, 1089–1100 (2023). DOI: 3.

4 Dauparas J. et al. "Robust deep learning-based protein sequence design using ProteinMPNN". *Science* 378,49-56(2022). DOI: 4.

5 Wu, R. et al. "High-resolution de novo structure prediction from primary sequence". *BioRxiv* (2022). DOI: 5.

6 Gainza, P., Sverrisson, F., Monti, F. et al. "Deciphering interaction fingerprints from protein molecular surfaces using geometric deep learning". *Nat Methods* 17, 184–192 (2020). DOI: 6.

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